

Amendments to the Specification:

Please replace the first paragraph that begins on page 21 of the specification with the following paragraph.

Surprisingly, our first attempt to find evidence that potentially problematic regions do exist, was successful and even more surprisingly, we obtained experimental confirmation by finding unexpectedly an improvement of orders of magnitude. An analysis of the sequence derived from the RNA virus of expression vector pICH8543 (EXAMPLE 1, Fig. 6A) using the Netgenell server program (<http://www.cbs.dtu.dk/services/NetGene2/>) (Hebsgaard *et al.*, 1991, *J. Mol. Biol.*, 220, 49-65) for the presence of cryptic introns and RNA splicing sites showed the presence of intron-like regions that might be spliced by the nuclear RNA processing machinery (see circled regions in Fig. 2). There are many other programs that can be used to identify potentially problematic regions (said selected localities) within plant viral RNA sequences, such as exon/intron prediction program (<http://genes.mit.edu/GENSCAN.html>) (Burge & Karlin, 1997, *J. Mol. Biol.*, 268, 78-94) or splicing signal prediction program (<http://125.itba.mi.cnr.it/~webgene/wwwspliceview.html>) SpliceView of ITB, the Italian Institute for Biomedical Technologies, for a variety of organisms.

Please replace the first paragraph that begins on page 33 of the specification with the following paragraph.

We analyzed the sequence of the RNA replicon from pICH4351 using the Netgenell server program (<http://www.cbs.dtu.dk/services/NetGene2/>) (Hebsgaard *et al.*, 1991, *J. Mol. Biol.*, 220,49-65) and noticed several intron-like sequence features that might induce alternative splicing events. One such feature is a 0.6 kb uridine-rich region (corresponding to nt 827 to 1462 in GenBank accession BRU03387) at the beginning of the RdRP (Fig. 2A). This region was replaced in pICH14833 by a PCR-mutagenized sequence that differs from the original sequence by a 54 nucleotide substitution (sequence given in the annex as SEQ ID No. 2; cf. Fig. 3). The 52 nucleotide substitutions were made to replace T-rich sequences by more GC-rich sequences. All nucleotide substitutions were made silent so as not to change the RdRP protein sequence. This mutagenized fragment also contains two nucleotide substitutions (at position 829 and 1459; coordinates relative to GenBank accession BRU03387) that were introduced to remove putative cryptic splice donor and acceptor sites, respectively. To test the effect of these mutations, the resulting clone pICH15466 (Fig. 6A) was agroinfiltrated in *N. benthamiana* leaves with or without pICH10745 (movement protein *in trans*). Eight days after infiltration, a 10-fold increase in the number of GFP expressing cells was observed in the area infiltrated with pICH15466 (compared to pICH14833, Fig. 7). This suggests that removal of intron-like sequences from the viral amplicon prevents unwanted alternative splicing events and results in more efficient initiation of viral replication. Coinfiltration of pICH15466 and pICH10745 leads to cell-to-cell movement of the modified replicon at a similar speed as a non-modified replicon. This shows that the modification of the RNA sequence did not affect cell to cell movement of the viral vector.

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Amdt. dated February 16, 2010
Reply to Office Action of August 19, 2009

Please replace the first paragraph that begins on page 38 of the specification with the following paragraph.

The analysis of RNA profile of selected plant RNA viruses as well as one well characterised plant gene (AtDMC1) was performed by using the Netgenell server program (<http://www.cbs.dtu.dk/services/NetGene2/>) (Hebsgaard *et al.*, 1991, *J. Mol. Biol.*, 220,49-65). The RNA profile shown in Fig. 9 for AtDMC1 clearly reflects the presence of 14 introns (circled), previously identified by comparing the cDNA and genomic DNA sequences. It is evident that RNA profiles of two plant viruses have regions (see the Figures 10, 11) which might cause problems for the stability of said RNA, if they are placed in plant nuclear environment. We have analysed the RNA profiles of several other representatives of plant RNA viruses (not shown), such as Brome Mosaic Virus, different strains of TMV, and many others. All of them have potential problematic regions that might compromise the efficiency of plant RNA virus-based replicon formation if delivered into the plant cell as DNA precursors.